

SECONDARY THICKENING IN GEOPHYTIC LILIACEAE

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ABSTRACT

Secondary thickening is known to be present in seven geophytic species of the Liliaceae, but this is much less pronounced than in the well known arborescent Liliaceae.

In the arborescent species secondary thickening starts at a considerable distance from the apex of the stem, whereas it is initiated relatively near to the apex of the stems of the studied species.

The amount of secondary tissue is small and more secondary parenchyma than secondary vascular tissue is formed.

Secondary vascular bundles originate when some of the primary bundles branch, one branch being the secondary bundle, the other a continuation of the primary bundle.

The secondary bundle (branch) may be due to the activity of an intrafascicular cambium in the primary bundles.

In old parts of the rhizomes the cambium is sometimes transformed into storage tissue.

INTRODUCTION

An idea which is prevalent among botanists is that secondary thickening in monocotyledons is limited to a few arborescent species of the Liliaceae. The related geophytic types have, however, not been fully investigated.

The only work which has been done on this problem in a related group in South Africa is that of Adamson¹ on the Iridaceae.

The following liliaceous species were examined during this study:

Bulbine coetzeei Oberm.

Anthericum cooperi Bak.

Anthericum fasciculatum Bak.

Trachyandra saltii (Bak.) Oberm.

Chlorophytum comosum (Thunb.) Jacques

Chortolirion stenophyllum (Bak.) Berger

Urginea lydenburgensis R. A. Dyer

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In the well-known representatives of the arborescent Liliaceae with secondary growth such as *Aloe*, *Dracaena*, *Cordyline* and *Yucca* (Anderson², Röseler¹⁰), the secondary tissue forms an important part of the stem. This is, however, not the case in the species studied. In these species the secondary tissue is of lesser importance on account of the limited amount formed.

Holm⁸ is of opinion that the secondary tissues in monocotyledons have two functions, firstly, to act as a "supporting apparatus" and secondly, to serve as tissue for the deposition of nutritive substances.

Support is, however, not necessary for stems or rhizomes of the plants studied, because of their compactness and subterranean habit.

As far as the storage tissue is concerned, starch grains were found in the older parts of the secondary tissue of *Trachyandra saltii*. Holm⁸ reports the occurrence of starch grains in the secondary tissue of *Dioscorea sativa*, so that this may not be altogether an uncommon occurrence.

The paucity of secondary tissues in the species studied is perhaps due to the shortness of the stems. Plants with short stems transport water and nutritive substances over relatively short distances only. Cheadle⁶ found that water is transported by the younger secondary xylem elements. In the species studied there is thus no "incentive" to form large amounts of secondary tissue.

Specimens of plants (usually in flower) were collected and fixed in formalin-aceto-alcohol. The stems or rhizomes were embedded in paraffin wax and serial sections made with a rotary microtome.

Sections were stained with Fast Green and Safranin. Most illustrations are, for the sake of clarity, combinations of drawings and photomicrographs.

MORPHOLOGY

Transverse sections of the rhizomes of the plants studied show that primary cell multiplication takes place just below the apex. This area is characterised by new cell walls laid down at random angles to the circumference of the rhizome.

Immediately below the apex procambial bundles are present (fig. 1). These bundles are embedded in the tissues of the primary meristem. In most species approximately six such procambial groups are formed. Large numbers of primary vascular bundles are present in the old parts of the rhizomes, but this small number of vascular initials are the only groups of vascular tissue which can unequivocally be referred to as being formed by the primary meristem.

Active primary cell multiplication is less evident in older tissues where the vascular bundles are differentiated into xylem and phloem.

In most of the named species a large number of calcium oxalate crystals are scattered throughout the parenchymatous tissues of the rhizome. These crystals are in the shape of raphides, or less often, druses. The crystals are abundant,

especially in the peripheral layers of the rhizome, but also appear in the rest of the tissues, even in the surrounding leaf bases. In *Chortolirion stenophyllum* approximately 4% of the parenchymatous cells contain crystals of calcium oxalate.

An annular ring which does not contain these crystals is present some distance below the apex, around the vascular system of the rhizome. This ring represents a band of meristematic cells which differentiate into a cambial band in older parts of the rhizome.

At no stage in the development of this cambium band are the meristematic initials present as a single layer of cells. As soon as the cambium band becomes visible, it is at least three cell layers in width (fig. 2).

An area in which no primary cell multiplication is evident, is present some distance below the apex of the rhizome. The cambial band becomes visible below this area. There is, therefore, a distinct cessation of primary growth before secondary growth is initiated. This is true in all the studied species.

The presence of this area in which no primary cell multiplication is evident, refutes the hypothesis of Carano (according to Cheadle⁶) that the primary and secondary meristematic regions are continuous.

The apices of secondary bundles are visible in the cambial band (fig. 3). These secondary bundles are continuously lengthened by the addition of procambial cells, originating in the cambial tissue.

When a series of transverse sections through the apical termination of a secondary bundle is studied, it is clear that more than one cambial cell takes part in the propagation of a secondary bundle at a specific height in the rhizome. This is in agreement with the findings of Lovén⁹, Adamson¹ and Esau⁷.

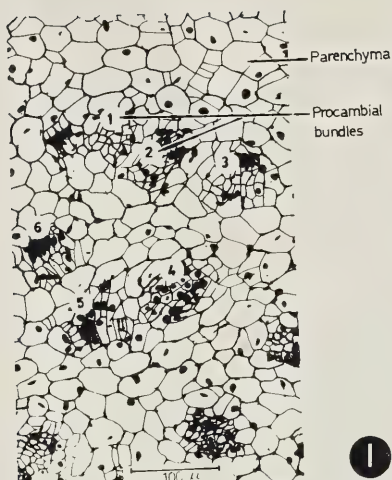
When a single section in which the apex of a young secondary bundle is situated, is studied, it is easy to misinterpret the propagation of such a young bundle and conclude that it is lengthened by the differentiation of the products of a single cambial cell, as Chamberlain⁵ suggests.

In older parts of the rhizome the secondary bundles originate at the point where a primary bundle branches (fig. 4).

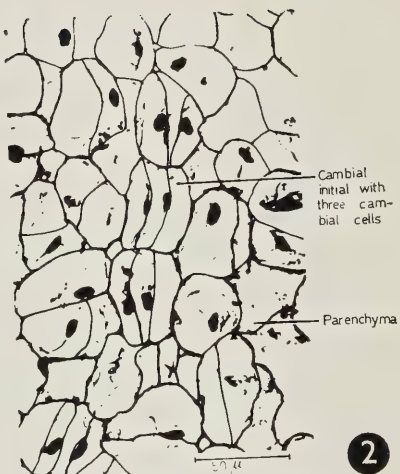
A primary bundle may branch several times within a relatively short distance, to give rise to successive secondary bundles, one being formed at each such branching.

It is not possible to state conclusively in which way a primary bundle gives rise to a secondary bundle. It is distinctly possible that a secondary bundle may develop from a primary bundle when the latter is not yet fully developed, by means of the intrafascicular cambium. This type of cambium was found in various primary vascular bundles of *Anthericum cooperi*, *A. fasciculatum*, *Chlorophytum comosum* and *Chortolirion stenophyllum*.

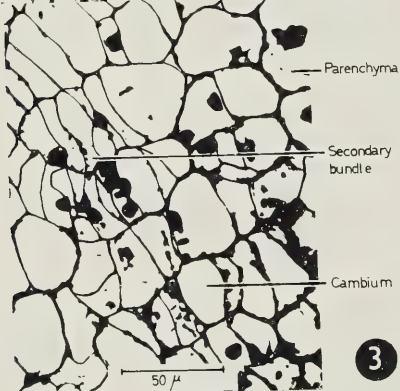
Especially in the case of (fig. 5) *A. fasciculatum* is the condition where a



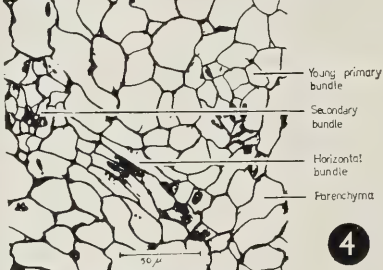
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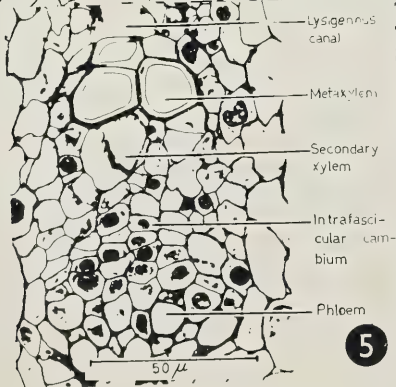
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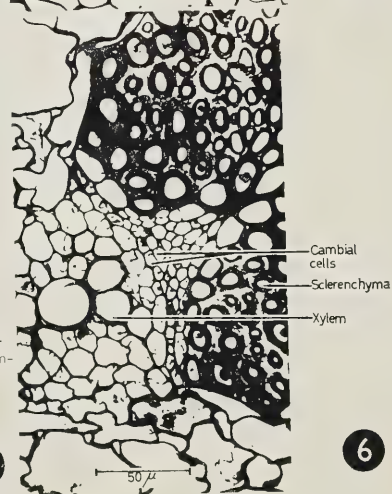
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secondary bundle may originate from the actively dividing intrafascicular cambium in a primary bundle strongly suggested.

Secondary bundles generally originate from primary bundles when the latter are fully or nearly fully differentiated. An intrafascicular cambium is present only in young primary vascular bundles (leaf traces), or in the procambial cell groups directly below the apex of the rhizome. In *A. fasciculatum* only is an intrafascicular cambium present in fully developed vascular bundles of old leaves (fig. 6). This seems to preclude an intrafascicular cambium from contributing to the formation of secondary bundles, but small numbers of such cambial cells may nevertheless be present in some of the primary bundles although not easily visible.

Arber³ states that the presence of an intrafascicular cambium in monocotyledons is a widespread anatomical feature, a remark which is to a large extent substantiated in this study.

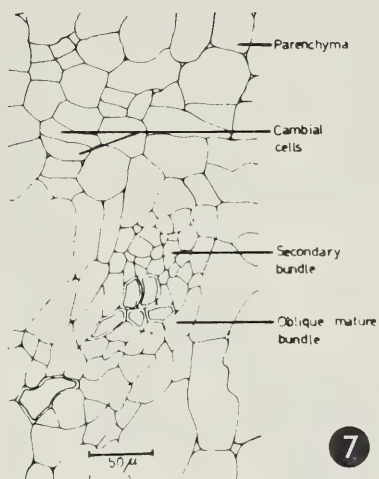
Arber⁴ found lateral vascular bundles in the leaves of an unnamed species of *Anthericum*, originating in the intrafascicular cambium of primary vascular bundles. Much more, however, remains to become known of the formation or origin of these secondary vascular bundles.

Numerous connections exist between the primary and secondary vascular systems of the rhizomes. In serial sections through the rhizomes or stems of all the species studied, no single secondary vascular bundle which did not originate during a branching of a primary vascular bundle was found. In all cases these secondary bundles terminate apically in the cambial band some distance below the apex of the rhizome.

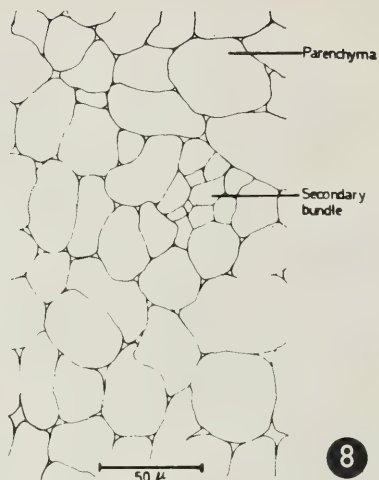
There seems to be a short distance only in which the cambial band is capable of forming secondary vascular bundles, because the apical terminations of such bundles are usually found in a rather short (less than 1.5 mm) vertical distance in the cambium. Below this "active" part of the cambium secondary bundles are also encountered, but no terminal points can be found, so that it can be inferred that no propagation of secondary vascular bundles takes place in this area.

In the "inactive" part of the cambium the secondary bundles may increase

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- FIG. 1. Transverse section of the rhizome of *Chlorophytum comosum*, showing six procambial groups of cells immediately below the apex.
- FIG. 2. Transverse section of the rhizome of *Trachyandra saltii*, showing the youngest cambial cells.
- FIG. 3. Transverse section of the rhizome of *Trachyandra saltii*, showing the apical termination of a secondary vascular bundle.
- FIG. 4. Transverse section of the rhizome of *Bulbine coetzei*, illustrating the branching of a primary vascular bundle.
- FIG. 5. Transverse section of the rhizome of *Anthericum fasciculatum*, showing the intrafascicular cambium in a primary vascular bundle.
- FIG. 6. Transverse section of the rhizome of *Anthericum fasciculatum*, showing intrafascicular cambial cells in an old primary vascular bundle.



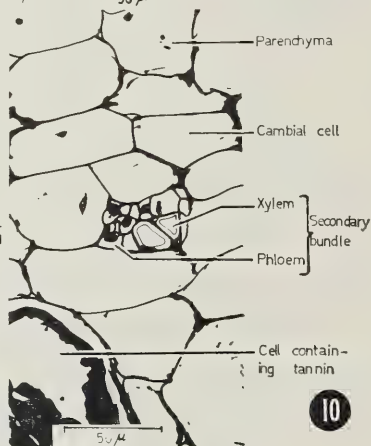
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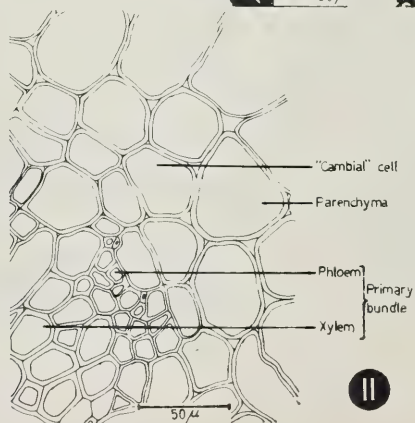
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slightly in diameter because of additional cambial cells which differentiate into secondary vascular tissue and are incorporated in the secondary bundles.

At the point where secondary bundles are at their oldest (just after emerging from a primary bundle) usually no differentiation into xylem and phloem is apparent (fig. 7). If differentiation has taken place, it is only to the extent of the formation of one or two xylem elements and a small number of sieve elements. No companion cells are formed.

Because of the undifferentiated state of the secondary bundles, even in a fully mature rhizome, these bundles cannot be very efficient transport pathways, either for water or organic substances.

It seems as if the cambial activity in these species is a rudimentary feature, reminiscent of the state found in the related arborescent Liliaceae, rather than an active mechanism for the formation of new transport facilities (secondary xylem and phloem) in the rhizome.

In *Chortolirion stenophyllum* a large number of secondary bundles may be found in a single transverse section of the rhizome, a short distance below the apex. At this distance from the apex the cambium is not present as a continuous band as yet in this species. It is only in the lower portions of the rhizome, where very few apical terminations of secondary bundles are present, that the cambium is visible as such a band.

The cambium in *C. stenophyllum* thus forms secondary vascular bundles when not fully differentiated (fig. 8) and, in the older state, secondary parenchyma only. The opposite is found in the other species, where the cambial band must be fully developed before secondary vascular bundles appear.

In all the species the amount of secondary vascular tissue formed is much less than the amount of secondary parenchyma produced.

Especially in *Trachyandra saltii* are the raphides of calcium oxalate present in all tissue of the rhizome, except in the cambial band itself. In the tissues between the cambial band and the epidermis, the raphides are oriented with their long axes approximately longitudinal to the long axis of the rhizome. In the tissues enclosed by the cambial band, the raphides are predominantly vertically oriented. No explanation for this observation can be tendered at present.

FIG. 7. Transverse section of the stem of *Urginea lydenburgensis*, showing the undifferentiated state of a secondary bundle at the point where it branches from a primary vascular bundle.

FIG. 8. Young secondary vascular bundle appearing before cambial band is differentiated in a transverse section of the rhizome of *Chortolirion stenophyllum*.

FIG. 9. Segmentation of the cambial band as seen in transverse section of the rhizome of *Trachyandra saltii*.

FIG. 10. Transverse section of the stem of *Urginea lydenburgensis*, showing a fully differentiated secondary vascular bundle.

FIG. 11. Transverse section of lower part of the rhizome of *Trachyandra saltii*, showing the thickened cell walls of the "cambium".

In most of the species there are indications of secondary vascular tissue produced by the cambium, taking part in the formation of adventitious roots. This can be seen in the younger parts of the rhizomes where adventitious roots are continuously being initiated. In these parts of the rhizome horizontal secondary vascular bundles appear next to the cambial band in the tissues between the cambium and the epidermis. These secondary bundles sometimes exhibit vascular cells in radial tiers, transversely across the length of the bundle. This is what is to be expected if these bundles are of secondary origin and differentiated laterally by a cambial layer. These vascular bundles appear prominently only where adventitious roots leave the rhizome, where the secondary bundles enter the adventitious root. In the process of entering the root the primary vascular bundles from the central parts of the rhizome break through the cambial band. This leaves semicircular segments of cambium around the vascular system of the rhizome, between the points where adventitious roots develop (fig. 9).

The secondary parenchyma does not seem to participate in the formation of adventitious roots, although it is difficult to determine exactly. This is due to the fact that much disruption of the regular orientation of cells is present at these positions. When the secondary parenchyma is not radially oriented, there is no way to distinguish it from the adjacent primary ground tissue.

The cambial band is recognisable as such only in the younger tissues of a rhizome. In the older parts it peters out, and is not visible a short distance above the oldest parts of the rhizome. This may be due to the limited formation of cambial tissues in the rhizome when very young, as well as to the disruption of regularly oriented tiers of cells because of the growth of surrounding tissues as the rhizome ages.

The maximum observed width of the cambial band (including the radially tiered secondary parenchyma) is approximately 12 cell layers. It is impossible to conclude in which specific cell layer the actual active meristematic cells are situated. It seems as if the idea of Schoute (according to Cheadle⁶) is correct. Schoute called the meristematic layer in monocotyledons an "Etagenmeristem". This meristem consists of cells which become meristematic, divide several times and then lose their meristematic character. The result of the activity of such an "etagenmeristem" would be the formation of a wide band of cells in which the true meristematic cells are distributed. Röseler¹⁰ also found such cambial initials in a sheath or band of nominally meristematic cells. This is the only way in which the observed multilayered cambium could have developed. This view is strengthened by the fact that the apical terminations of young secondary bundles may be situated at different distances from the periphery of the cambial band.

In *Urginea lydenburgensis* a few secondary vascular bundles are present in the lowest parts of the cambial band. These secondary bundles differ from the

other secondary bundles encountered in that the xylem appears to be fully lignified and the phloem consists of cells with appreciably thickened walls (fig. 10). In this case the cambial cells are thin walled with large nuclei and still appear to be capable of cell division, although the cambial band is limited to a very few cell layers in width. The connection between these secondary bundles and the primary vascular system could not always be found, because of the extreme complexity of the vascular system in the oldest parts of the stem.

A rather similar condition is found in the lower parts of the rhizome of *Trachyandra saltii*, but in this case very few if any secondary bundles are present in the older parts of the rhizome. The cambial band, however, consists of cells with thickened cell walls, which should preclude cell divisions from taking place (fig. 11). Only in isolated parts of the "cambium" are the cells in radial tiers. In all other parts of the "cambium" the radial orientation of the cells is disrupted by the pressure of surrounding tissues. Starch grains are present in these cells, so that the cambium fulfills the functions of storage, rather than meristematic tissue.

In the younger parts of the cambial band of most species, primary leaf traces are present. These leaf traces are in some cases nearly completely undifferentiated and resemble secondary bundles closely. The only way in which they may be distinguished from secondary bundles is by following them from section to section to their upper and/or lower extremities. They appear to be present in the cambial band incidentally only, because other leaf traces, to all appearances exactly similar, are present both outside and inside the cambial band.

Peridermal tissue is present on the exposed surfaces of the rhizomes of most species. In *Anthericum cooperi* the parts of the rhizome which are protected by the old leaf bases are covered by a thickened and lignified epidermis, whilst those parts not protected by leaf bases are covered by a periderm of approximately three cell layers thickness.

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